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Comparative *in vitro* activity of garenoxacin against *Chlamydia* spp.

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The *in vitro* susceptibilities of 33 isolates of *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittaci* to a new quinolone drug, garenoxacin (BMS-284756), in comparison with levofloxacin, ciprofloxacin, doxycycline, erythromycin and roxithromycin, were determined. Garenoxacin was the most active of the quinolone drugs tested, with identical MIC and MBC, which ranged from 0.007 to 0.03 mg/L. The MIC and MBC of the other two quinolones tested, levofloxacin and ciprofloxacin, were also identical, ranging from 0.25 to 2 mg/L. The MICs and MBCs of doxycycline, erythromycin and roxithromycin were also determined.

Introduction

Members of the genus *Chlamydia* are obligate intracellular parasites that play an important role in both human and animal diseases.¹ *Chlamydia trachomatis* causes human genitourinary tract infections and is associated with neonatal conjunctivitis and pneumonitis. *Chlamydia psittaci* strains are related to human respiratory tract infections acquired from infected animals. *Chlamydia pneumoniae* is a relatively new species, which causes respiratory tract infections in humans. *Chlamydia pecorum* strains are isolated from ruminants. Although tetracyclines and macrolides were recommended as first-line drugs for the treatment of chlamydial infections in humans, the development of effective antimicrobial agents for the treatment of chlamydial infections is very important, in order to prevent the frequent recurrence of these infections. The new fluoroquinolones have potent *in vitro* activity against a broad range of atypical respiratory pathogens, including *Legionella*, *Chlamydia* species² and *Mycoplasma pneumoniae*.³ In the present study, we tested the activity of a new quinolone, garenoxacin (BMS-284756), against *C. trachomatis*, *C. psittaci* and *C. pneumoniae* strains in comparison with the quinolone drugs levofloxacin and ciprofloxacin, and with doxycycline, erythromycin and roxithromycin.

Garenoxacin is a des-F(6) quinolone (lacking the 6-position fluorine typical of existing fluoroquinolones) with a broad range of antibacterial activity against Gram-negative and Gram-positive pathogens, including certain quinolone-

resistant strains.⁴ This des-F(6) quinolone exhibits activity against respiratory pathogens and could be a therapeutic option for empirical treatment of respiratory tract infections. The high intrinsic *in vitro* activity against fastidious and atypical microbial species such as mycoplasmas³ or ureaplasmas, *Legionella* spp., *Helicobacter pylori*,⁵ *Borrelia burgdorferi*, *C. trachomatis* and *C. pneumoniae* strains and gonococci,⁶ supports its use for the treatment of community and nosocomial infections.

Garenoxacin has the same mechanism of action against DNA topoisomerases as other quinolones but has a stronger level of potency.⁶ In fact, its antibacterial activity and spectrum compared with five quinolones (trovafloxacin, moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin) was found to be high against a significant number of Gram-positive and Gram-negative, aerobic and anaerobic bacteria.⁶

Materials and methods

Bacterial strains and growth conditions

A total number of 33 *Chlamydia* isolates were tested: 10 *C. trachomatis* isolates, three *C. pneumoniae* isolates and 20 *C. psittaci* isolates. Isolates of *C. trachomatis* included five typed strains (serovars D, E, H, I and LGV2) and five untyped clinical isolates recently isolated from urethral swabs of male patients with non-gonococcal urethritis. *C. pneumoniae* isolates comprised one recent isolate from Italy and two reference isolates: 10L-207 (originally isolated in 1967 in London

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from an Iranian child with trachoma) and CM-1 (a clinical isolate from the Centers for Disease Control and Prevention, Atlanta, GA, USA, ATCC VR-1360) serologically identical by the micro-immunofluorescence test to the reference isolate TW183 (ATCC VR-2282). The 20 isolates of *C. psittaci* studied included eight reference strains and 12 isolates from infected animals in Italy (provided by Dr Magnino, Diagnostic Section, Zooprophyllactic Institute, Pavia, Italy). The eight reference strains were represented by the avian (parakeet) strain 6BC (USA), the ovine (lamb) strain A22 (Scotland), the ovine strains W73, S26/3 and P787 (Scotland), the caprine abortion strain Krauss 15 (Scotland), the feline pneumonitis strain FePn-145 (USA) and the meningopneumonitis strain MePn-343 (ferret, USA). Susceptibility testing of the 33 chlamydial isolates tested was performed in LLC-MK2 cells (a continuous cell line derived from Rhesus monkey kidney tissue) grown in 24-well plates containing a glass coverslip (diameter, 12 mm) at the bottom. The cell growth medium was Eagle's minimum essential medium (EMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 10 mg/L gentamicin, 10 mg/L vancomycin and containing 2 mM glutamine and 1.7 mg/L glucose.

Antimicrobial drugs and sensitivity assays

The antimicrobial drugs were provided as powders and solubilized according to the instructions of the manufacturers. Garenoxacin was supplied by Bristol-Myers Squibb (Syracuse, NY, USA), levofloxacin was purchased from Ortho-McNeil Pharmaceutical (Mt Prospect, IL, USA), ciprofloxacin was supplied by Bayer (West Haven, CT, USA), and doxycycline, roxithromycin and erythromycin were purchased from Sigma (Milan, Italy). Each of the 24-well plates was inoculated with an inoculum of chlamydiae that yielded 5×10^1 inclusion forming units (ifu) per millilitre and centri-

fuged at 1700g for 1 h. The medium was then removed and replaced with EMEM supplemented with 10% FCS, glucose (5 mg/L), cycloheximide (1 mg/L) and serial two-fold dilutions of each antibiotic. All tests were run in triplicate. After incubation at 35°C for 48 h, cultures were fixed with methanol and stained for inclusions with a fluorescein-conjugated monoclonal antibody specific for the chlamydial lipopolysaccharide genus-specific antigen.⁷ The MIC was the lowest concentration preventing >90% chlamydial inclusion detection compared with the drug-free control. The minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic-containing medium, washing wells twice with phosphate-buffered saline, adding antimicrobial-free medium and re-incubating the plates for 48 h at 35°C. Cells were then fixed and stained as described above. The MBC was the lowest antibiotic concentration resulting in >90% reduction of inclusions.

Results

The MICs and MBCs for *C. trachomatis* and *C. psittaci* are shown in Table 1. The MICs and MBCs for *C. pneumoniae* are shown in Table 2. Both the MICs and MBCs of garenoxacin for the 33 *Chlamydia* isolates ranged between 0.007 and 0.03 mg/L. The MIC values of levofloxacin and ciprofloxacin were the same as the MBCs, ranging between 0.25 and 1 mg/L and between 1 and 2 mg/L, respectively. The MICs of doxycycline ranged between 0.03 and 0.06 mg/L, and the MBCs ranged between 0.06 and 0.125 mg/L. The range of MICs of erythromycin was between 0.125 and 1 mg/L and MBCs ranged from 0.25 to 2 mg/L. The MICs of roxithromycin ranged between 0.06 and 0.25 mg/L and the MBCs between 0.125 and 0.5 mg/L.

Table 1. MICs (mg/L) and MBCs (mg/L) of garenoxacin and other antimicrobials for 30 isolates of *C. trachomatis* and *C. psittaci*

Agent	Organism	No. of isolates	MIC range	MIC ₅₀	MIC ₉₀	MBC range	MBC ₅₀	MBC ₉₀
Garenoxacin	<i>C. trachomatis</i>	10	0.007-0.03	0.015	0.03	0.007-0.03	0.015	0.03
	<i>C. psittaci</i>	20	0.007-0.03	0.015	0.03	0.007-0.03	0.015	0.03
Levofloxacin	<i>C. trachomatis</i>	10	0.25-1	0.5	0.5	0.25-1	0.5	1
	<i>C. psittaci</i>	20	0.25-0.5	0.5	0.5	0.25-0.5	0.5	0.5
Ciprofloxacin	<i>C. trachomatis</i>	10	1-2	2	2	1-2	2	2
	<i>C. psittaci</i>	20	1-2	1	2	1-2	1	2
Doxycycline	<i>C. trachomatis</i>	10	0.03-0.06	0.03	0.06	0.06-0.125	0.06	0.125
	<i>C. psittaci</i>	20	0.03-0.06	0.03	0.06	0.06-0.125	0.06	0.125
Erythromycin	<i>C. trachomatis</i>	10	0.125-1	0.5	1	0.25-2	1	2
	<i>C. psittaci</i>	20	0.125-0.25	0.5	0.5	0.25-1	1	1
Roxithromycin	<i>C. trachomatis</i>	10	0.125-0.25	0.25	0.25	0.25-0.5	0.5	0.5
	<i>C. psittaci</i>	20	0.06-0.25	0.125	0.25	0.125-0.5	0.25	0.5

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Table 2. MICs (mg/L) and MBCs (mg/L) of garenoxacin and other antimicrobials for three isolates of *C. pneumoniae*

Isolates	Garenoxacin		Levofloxacin		Ciprofloxacin		Doxycycline		Erythromycin		Roxithromycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
FB	0.015	0.015	1	1	2	2	0.06	0.125	0.25	0.5	0.125	0.25
CM-1	0.015	0.015	1	1	1	1	0.06	0.125	0.25	0.5	0.125	0.25
IOL-207	0.007	0.007	0.5	0.5	2	2	0.06	0.125	0.25	0.5	0.125	0.25

Discussion

Chlamydia spp. are human pathogens that cause respiratory and genital tract infections worldwide. Tetracycline and macrolides have been used frequently for the treatment of chlamydial infections. Recently, quinolones have attracted interest for their potential use in the therapy of respiratory infections. *In vitro* studies have also demonstrated that some fluoroquinolones are active against chlamydial species.²

In this study, the activity of the new quinolone, garenoxacin, has been tested *in vitro* against *Chlamydia* spp. in comparison with other quinolones, and with doxycycline and the macrolide drugs, erythromycin and roxithromycin.

Garenoxacin was the most active of the quinolone drugs tested, with MICs and MBCs ranging from 0.007 to 0.03 mg/L. The concentration of garenoxacin at which 90% of the isolates were inhibited and killed was 0.03 mg/L for *C. trachomatis* and *C. psittaci* isolates. The MICs and the MBCs of garenoxacin ranged from 0.007 to 0.015 mg/L for the three *C. pneumoniae* isolates. The MICs and MBCs of garenoxacin were consistent from species to species and from strain to strain. These results are very similar to those reported recently by Malay *et al.*⁸ who tested five strains of *C. trachomatis* (MICs and MBCs of 0.015 mg/L) and 20 recent clinical isolates of *C. pneumoniae* (MIC₉₀ and MBC₉₀ of 0.015 mg/L).

The finding that for the 33 chlamydial isolates studied the MICs of garenoxacin, levofloxacin and ciprofloxacin were the same as the MBCs confirms previous observations that quinolones, at concentrations equivalent to their MICs, seem to kill chlamydial strains.^{9,10} Our MIC data were also similar to a previous finding.¹⁰ Malay *et al.*⁸ reported that garenoxacin was the most active quinolone against *C. pneumoniae* and *C. trachomatis* isolates tested. Our data confirm those results. In addition, our data also demonstrated that garenoxacin was the most active quinolone against *C. psittaci*, therefore demonstrating that garenoxacin is the most active quinolone tested thus far against chlamydiae.

Finally, it is noteworthy that in this study there was no significant variation in antimicrobial sensitivity among strains from the various chlamydial species and several

passages of the chlamydial strains in cell culture did not modify their sensitivity to antimicrobial drugs.

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